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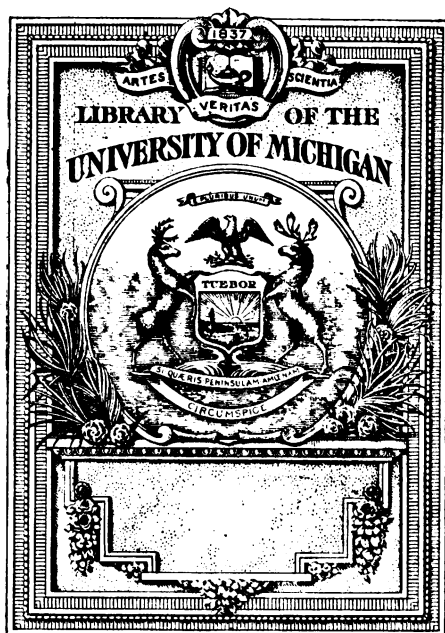
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Some New Bacterial Diseases of  
Legumes  
and  
The Relationship of the Organisms  
Causing the Same

THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL OF  
THE UNIVERSITY OF PENNSYLVANIA IN PARTIAL FUL-  
FILMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF DOCTOR OF PHILOSOPHY

BY  
THOMAS F. <sup>Franklin</sup> MANNS

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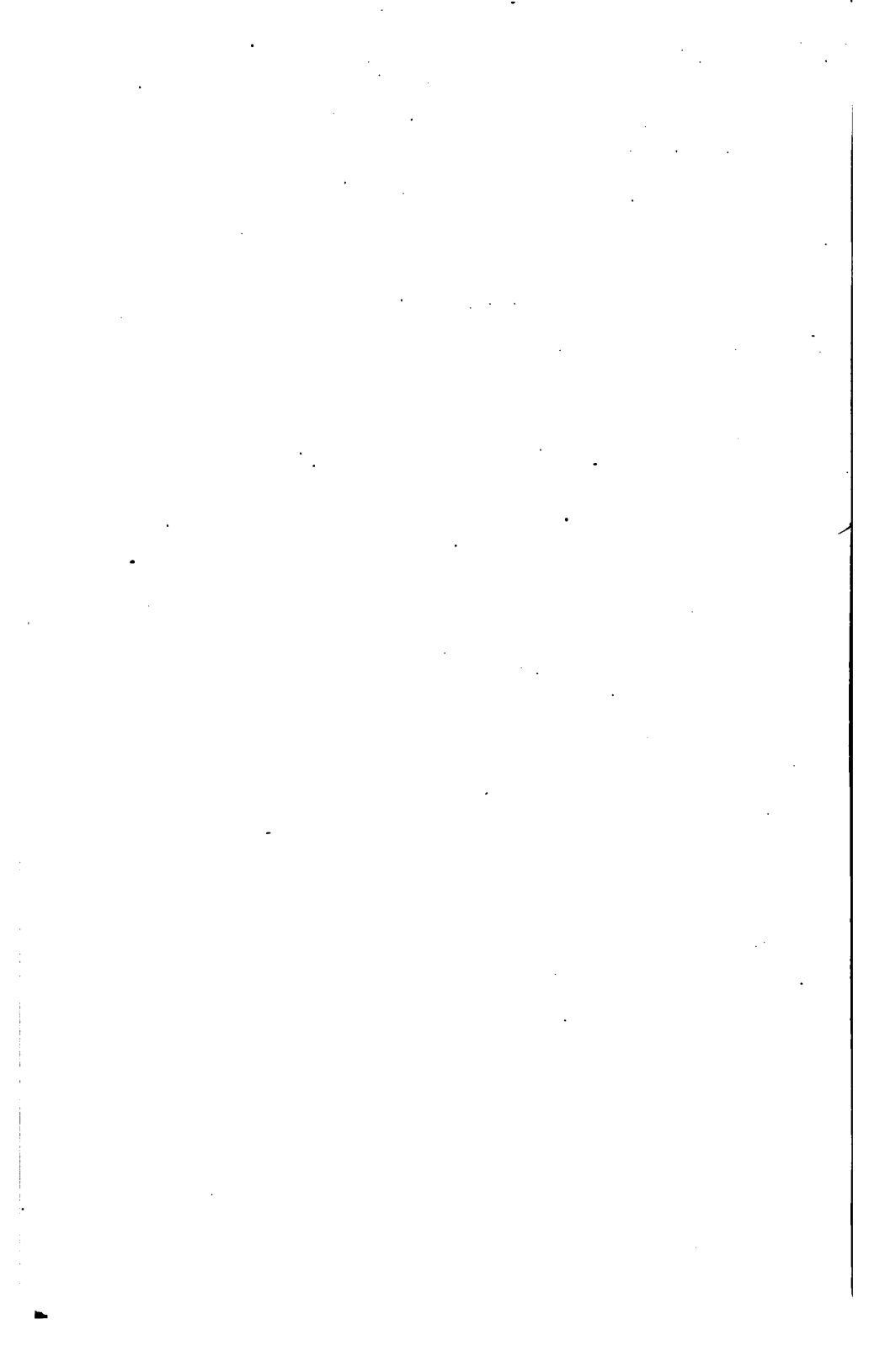
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**Some New Bacterial Diseases of Legumes and  
the Relationship of the Organisms  
Causing the Same**

**BY THOMAS F. MANNS**

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**Newark, Delaware**



# Some New Bacterial Diseases of Legumes and The Relationship of the Organisms Causing the Same\*

By THOMAS F. MANNS

## INTRODUCTION

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The history of phytopathology in relation to bacterial diseases is of comparatively recent years. We are indebted to Burrill<sub>1</sub> (1877 & 1878) for the first contribution giving definite experimental evidence that bacteria may and do cause injuries to plants of a pathological nature. His work was done upon the twig blight of pear (1878-1883). Following closely upon the work of this investigator we find others demonstrating what some considered an impossibility, viz. that plants could be subject to bacterial diseases (See p. 27, Lehrbuch, Hartig. 1882)<sub>2</sub>. Among Burrill's contemporaries who were at work on bacteria that are active pathogens of plants may be mentioned Prillieux<sub>3</sub> (his first publication in 1879), Comes<sub>4</sub> (1880), Sorauer<sub>5</sub> (1886), Savastano<sub>6</sub> (1887), Arthur<sub>7</sub> (1886), Beyerinck<sub>8</sub> (1888), and Wakker<sub>9</sub> (1883). Erwin F. Smith<sub>10</sub> 1899-1901, has been active in removing the doubt existing on the possibility of bacteria being active producers of disease in plants. His painstaking and exact work on bacteria as causes of plant disease will stand as a monumental contribution to the science of plant pathology. Within the last decade the number of exact workers in the bacterial pathology of plants has increased rapidly; it is beyond the scope of this paper to mention their names. It is sufficient to state that we must admit that certain diseases which were at different times assigned to higher fungi as casual organisms are now known to be caused by bacteria.

This bulletin deals with a disease of the sweet pea (*Lathyrus odoratus*) which has variously been stated to be due to different parasitic fungi. It describes also similar diseases upon the clovers (*Trifolium* pp.), culinary beans (*Phaseolus* spp.), and soy bean (*Soja* spp.), which are here proved to be bacterial in nature.

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\*Submitted for publication June, 1913. Presented before the graduate school of the University of Pennsylvania as a thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1913.

## DEVELOPMENT OF PROBLEM

Mr. J. J. Taubenhau<sup>11</sup>, having taken up in 1911 the project of "Some Fungous Diseases of the Sweet Pea" for thesis work at the University of Pennsylvania, met a disease of the sweet pea known generally in England and less in the United States as "streak." Specimens of diseased plants from Boston, Mass., and from England were referred to the writer. The appearance of the disease suggested to him that it was of bacterial origin. Preliminary isolation work for the parasite readily revealed an abundance of bacteria in the beginning lesions. Attempts at infection of sweet peas with pure cultures gave positive results in ten days.

The symptoms of this "streak" disease of the sweet pea were so similar to those of a disease we had previously observed on red clover that it occurred to us that the latter was possibly of bacterial origin and related to the former. A search in the fields in the vicinity of Newark, Del., revealed the presence of the formerly observed disease, in red, alsike, and mammoth clovers. The "streak" disease was also prevalent on several species of *Lathyrus*, the latter of forage types and upon the numerous varieties of sweet peas with which Mr. Taubenhau was working. Platings of beginning lesions from the clovers, the different forage species of *Lathyrus*, and the different varieties of sweet peas indicated the causative organism to be a bacterium.

## HISTORY OF "STREAK"

The disease was first observed, according to a letter in 1912 from Mr. T. A. Weston of Orpington, England, by H. J. R. Digges of Dublin in 1904 or 1905. Mr. Weston states that, in the fall of 1906, he had a note of the disease in "The Gardener" describing it as "a new disease under the title of 'streak'." In 1908 George Massee<sup>12</sup>, in a letter to a correspondent who had sent in diseased specimens replied, "the disease is of a physiological nature."

As far as we have been able to obtain literature, it appears that only since the summer of 1908 has any definite work been done upon the disease. Chittenden<sup>13</sup>, in England during 1908, 1909 and 1910 carried out inoculation work with the supposed parasite, viz., *Thielavia basicola*, obtaining during the first two seasons negative results.

In this work Chittenden<sup>14</sup>, gave a good description of the disease. He found in 1910 that by excessive watering he was able to get infections from *Thielavia*, but he did not indicate that they were the typ-



ical "streak." He writes, "To sum up, as far as our experiments go, the "Streak" disease is brought about by the attack of the fungus *Thielavia basicola* on plants that have received some check at the root."

During the season of 1912 Massee<sup>15</sup> again took up the disease, and attributed it to *Thielavia basicola*.

Another worker in England, a Mr. Dyke<sup>16</sup>, of St. Margarets found *Macrosporium solani* constantly associated with the disease, and believed it to be the cause of the trouble.

The serious nature of the disease may be seen by noting the numerous popular articles which have appeared in various horticultural and gardeners' journals of England. Citations from several of the more important of these articles are as follows:

In the Fruit-Grower, Fruiterer, Florist and Market Gardener, p. 155 (England) of Sept. 5, 1907, Mr. T. A. Weston in the "*Kent Notes*" writes:

"I regret to note how the stripe disease, *Peronospora viciae*, increases every year, and this season it has been very prevalent everywhere. One always knows the tell-tale streaks in the foliage and vine, the stripes appearing in the flowers in due course."

In the Oct. 24, 1907 number of the same journal Mr. Weston again writes upon the same disease, partly quoting a letter from a friend in Ireland.

"Mr. B— has the same thing in freshly broken up grazing land, no manure of any kind being used. Haulm 7-8 in. wide on healthy plants on same ground."

He goes on to say that he sent a couple of plants to Mr. Massee, of Kew, who gave it as *Peronospora trifoliorum*. That was two years ago. This year he has had an expert watching haulm and foliage under a microscope since the disease showed itself, the plants at that time being 6 in. Whole plants 6 ft. have been lifted and grown under conditions that would have brought fungoid disease out if it existed, yet no trace of such has been discovered either in the tissues or externally. The roots of diseased plants all showed very poor root development, few fibres and total absence of nitrogenous nodules. Whole batches of some sorts were affected, while with others a plant here and there showed the effects. Some showed the trouble before a flower appeared, others gave some beautiful flowers and then went wrong.

"So far, my friend. Now, this is as I myself find it. A lot of

my plants are going off, and I am sending you samples of growth, roots and flowers. Is it an atmospheric disease, soil disease, eelworm, or carried over in the seed? A friend of mine near London also reports an awful time this year, and he cannot get any really useful advice. I've seen journal experts referring to it as stated by Massee, but I am beginning to doubt it very much. My Irish friend has sprayed with copper without avail. Kent T. A. W."

In "The Gardener" for Nov. 16, 1907, p. 585, Mr. Weston gives a somewhat more detailed statement of the disease. The article is here quoted in full:

### THE STREAK DISEASE OF SWEET PEAS

"I fully expected that this steadily increasing trouble would have been discussed by the many able exponents of Sweet Pea culture connected with The Gardener.

"My note on p. 419 brought me several letters from a gentleman well known by name to the sweet pea world, and I venture to quote a few extracts, as they tend to show how serious the trouble is. The writer, an amateur by the way, says, 'I have had the disease in my garden for years, and it has been worse than ever this year, although most of my Sweet Pea plots have been replaced with fresh top spit loam from grazing land. I put 20 tons of this soil in a 60-foot row. A friend also broke up a new piece of pasture, giving nothing in the way of manure, not even artificials, and his plants were equally as bad as my own. Plants that escaped the disease were tremendously vigorous. I am of the opinion that the Peronospora theory is played out, for this year I had many plants carefully tested under the microscope, both root and branch, throughout the season, and not a trace of fungus was to be found. Some plants showed the disease, or whatever it is, before any flowers developed, others gave a few blooms and then went wrong. I am so disheartened that I think of giving up Sweet Peas altogether.'

"The foregoing tends to show that there is danger in this disease. As I have previously stated, I have seen the trouble for years, but never looked upon it as serious until two years ago, when it prevented my cutting any quantity of King Edward VII. This year I found it decidedly serious, more so when a gentleman visiting the National show told me that nearly all his plants were ruined by it.

"With us two or three clumps showed the disease early in the season, while single plants in other clumps also gave evidence of the

trouble, and so seriously were they affected that I pulled up the plants. One I particularly noticed, because it was the only one remaining of the variety. This showed a brown mark a few inches above the ground, the plant then being about 1 yard high. In a few days the brown mark had extended and had totally encircled the main stem, and also one of the laterals adjoining, while other laterals also showed signs of attack. The badly affected lateral went off limp, and on cutting it away I found the tissues, where affected by disease, quite dead. So alarmed was I that I scraped with a knife the outer covering of the main stem where affected, and could not find any live or green tissue, although the plant was still healthy. On close examination I found a plant of another variety similarly affected, and in utter hopelessness I painted the affected parts with strong Bordeaux mixture. The single plant got no worse, and eventually became a giant specimen, the other collapsed.

“Later on came the streaked foliage amongst many plants, while streaked, starved looking flowers were produced from all affected plants. The disease did not confine itself to any one variety, nor to every plant of one variety. Nigger, Midnight, Lord Nelson, Hetty Green, Helen Pierce, Frank Dolby, Enchantress, John Ingman, Cod-sall Rose, and Lady Pollock all gave us streaked flowers, and although the affected plants continued to grow and flower right up to October, they never recovered normal health; in fact, as the season advanced the flowers got worse, until it was impossible to find a flower worth a second glance. It has been declared to be the Vetch disease, *Peronospora trifoliorum*, and sulphide of potassium, or sulphurated potassium has been given as the remedy. A local grower found this of no value whatever, whilst the writer of the letters referred to has tried everything possible without result; and the fact of his being unable to discover disease spores in any shape or form shows it to be useless to use spray fluids. Another well known grower, however, informed me that after spraying with ‘sul. potass.’ his plants sent out new growth unaffected by the trouble.

“A peculiarity about the diseased plants is their poor root development, nodules being totally absent. Personally I am doubtful as to the trouble being due to *Peronospora*. Unlike the Sweet Pea blight, the disease under notice does not confine itself to the foliage at the start, but seems to attack the whole plant at once. The stems show brown streaks, as do the leaves, whilst the tops of the growths

are curled and twisted. The flowers are distorted and look quite out of character. Is the disease a product of California? Is it a bacterial disease? Has it any affinity to curl disease in Potatoes? If it comes through the seed, why are not all the plants from one packet affected? Advice is urgently needed by growers both in England and Ireland. Who can aid us? T. A. W."

In the February (1908) issue of the "Amateur Gardening" (England) p. 643, the "Streak" disease is again described as follows:

### "STREAK IN SWEET PEAS"

"Sweet pea growers in many parts of the country have had to contend with a comparatively new disease called the "Streak," which threatens to considerably hamper the successful growth of this popular flower.

"This disease appears in the form of greyish or brownish streaks, either on the plants when young or when in flower. The effect of an attack is the loss of natural color in the foliage and a sickly diseased appearance. In some cases whole groups of rows of plants have been quite ruined by the disease.

"So far its origin is more or less of a mystery, and all attempts to find a satisfactory remedy for it have resulted in failure. A peculiarity of the disease, apart from the streaks on the foliage, is the absence on attacked plants of the usual bacterial nodules on the roots. This naturally leads to the inference that a cold and wet season like that of last year may have prevented the nitrogen-fixing bacteria doing their work.

"The special appearance of the foliage caused by this disease must not be confounded with the grey and streaky results following an attack of thrips, or by sun scalds. The former is accompanied on the under side of the leaves by black shining dots; the latter is only partial. The Peaman"

In the Aug. 19th (1911) number of the "Amateur Gardening" the seriousness of the disease is clearly brought out as follows:

### "SWEET PEA DISEASE"

"As stated in a recent note, the streak disease has once again put in an appearance, and I am fain to confess that it has proved more virulent and destructive than ever before. In a season like last, the disease was somewhat slow in accomplishing its deadly work; in fact

the agony was long drawn out, for the plants continued to live for varying lengths of time, although incapable of producing satisfactory flowers. This season, however, there has been no mistaking its action, and one can only assume that the great heat has materially assisted the disease to accomplish its purpose. During the eight years that I have been acquainted with this disease, I have never known it to work such havoc as it has done this season, and now that five-sixths of my plants are wiped out beyond recall I feel I must record a few impressions.

"I do not for one moment suppose that I am relating a solitary case. More than one expert grower informed me that they were unable to show at the National owing to the disease. One of these gentlemen has won championship cups in previous years, and he assured me his plants had collapsed by the cartload. This tale was told me at the Olympia Show. At the Sweet Pea Show, no less than six expert gardeners from one district in Kent were upon my heels for a remedy. The disease was worse with them than ever before. At the sweet pea outing one or two efforts were made to secure some information, but the end of it all was nothing.

"What does it mean? Does it mean total annihilation of sweet peas? Plants affected by the disease do not produce seed. The results of my planting upon manured soil suggests that the disease comes to overfed plants, but the fact remains that streak can and does affect some plants on unmanured soil. It was visible at the N. S. P. S. trials, where no coarse growth is encouraged. I have seen it in the fields where sweet peas are growing for seed. It is not infrequently seen in America, for this much I elicited from one of our recent American visitors." "The Peaman."

Thruout the season of 1911 and 1912 discussions of a popular nature upon the "streak" disease of the sweet pea are found in many of the gardening and horticultural journals. The articles in the Amateur Gardening, The Gardeners' Chronicle and the Annual Reports of the National Sweet Pea Society, throw much light upon the nature of the disease.

The writer<sub>17</sub> presented a paper illustrated by lantern slides before the American Phytopathological Society at Cleveland on Jan. 3 of this year giving a preliminary report on his findings about this disease. The causal organism was announced as a newly described pathogen under the name *Bacillus lathyri*.

Directly following the above paper, W. Bateson read a paper by Dorothy M. Cayley,<sup>18</sup> before the Royal Society (London) under the caption "A Preliminary Note on a New Bacterial Disease of *Pisum sativum*" in which is described a disease having symptoms resembling in many respects those of the "streak" disease of sweet pea.

I quote from her paper the following:

"Investigations have been carried out this year at the John Innes Horticultural Institution to elucidate the nature of a disease which affects culinary peas (*Pisum sativum*).

"The disease, in this district at all events, is a serious one, killing a large proportion of the crop, but I have no information as to its prevalence in other parts of the country. I have succeeded in proving that the disease in culinary peas is caused by a large bacillus which exhibits a peculiar feature, inasmuch as it is transmitted in the interior of the seeds of the plant. As far as I am aware no analogous instances are known.

"The general symptoms are as follows: In mild cases after germination the shoot can develop normally, but in bad cases it is frequently abortive, brown and dead at the tip, and laterals grow out prematurely to take the place of the main shoot. Quite early in the development of the plant, when the plumule is from half an inch and upwards in length, light brown longitudinal streaks can be seen on the stem and root, and the first leaves are often brown at the tip. These streaks develop later into slits. In very bad cases little or no germination takes place. After this stage no further definite signs are noticeable till about the flowering period. Then the development of the disease depends a good deal on external conditions. If the weather is warm and dry, and the plants are growing vigorously, the disease develops rapidly, and in a few days the plants become unhealthy and change colour. The stem turns slightly brown, and looks somewhat water-soaked. Brown longitudinal streaks appear at the base of the petioles on either side of the rib of the stem, which is continuous with the mid-rib of the leaf. The streaks split open and dry out. The collar may be badly disorganized. The leaves become spotted, streaked and yellowish in color, and if the disease is progressing rapidly the younger portions of the plant show discoloration, and fail to develop properly.

"Except in bad cases the plants grow to full height, and can flower and set a certain amount of seed, but on examination the cotyledons of the seeds of a diseased plant show brown discoloration, which

may be limited to a mere spot in the centre of each cotyledon, or, on the other hand, nearly the whole of the cotyledon may be involved. In the latter case there is often a cavity in the centre of the cotyledon.

"Sections of the diseased cotyledon show large numbers of bacilli in various stages of development in the cells and intercellular spaces.

"The bacillus works its way into the intercellular spaces and then breaks into the cells. There the nucleus is often attacked, the cytoplasm destroyed, and the cells collapse, thus forming rents in the tissues.

"There is considerable evidence to show that the bacillus passes up the plant through the tissues above mentioned, through the funicle, and probably the micropyle into the young developing seed. If one pea is diseased all the other peas in the same pod are diseased to an equal extent. The disease is chiefly spread by the seed, but fresh infection may take place through the soil.

"Inoculation experiments were carried out in the open, but little stress can be laid on the results, as the disease was so prevalent throughout the experimental plot. Pea plants grown in heated soil in boxes, and inoculated just above the ground, when the plants were about 1 foot in height, showed no disease, whereas, in the open, seven out of ten inoculations on the stem just below the youngest unfolding leaf were successful.

"Further inoculation experiments are necessary, but the above results tend to show that the bacillus can only penetrate very young tissue. This is supported by the fact that large numbers of the bacilli have been found in the inner tissues of the radicle when only about half an inch long.

"Further investigations are in progress.

"In many respects the symptoms resemble those of the formidable disease of sweet pea (*Lathyrus odoratus*) known as "streak." This disease has been held to be due to *Thielavia basicola*, but, in view of these observations, that conclusion seems very doubtful, and I may add that, in the stem of diseased sweet peas, I have already found bacteria like those here described."

The fact that Miss Cayley has isolated bacteria from the stems of sweet pea affected with "streak" has caused her to question the conclusions of Massee and Chittenden, viz. that *Thielavia basicola* is the cause of "streak."

Beginning with the issue of Apr. 5 (1913) of the Gardeners'

Chronicle, the writer and his associate Mr. J. J. Taubenhaus published several articles giving a review of the diseases of the sweet pea (*Lathyrus odoratus*) to date.

The first article in the issue above noted describes and illustrates the "streak" disease.

### SYMPTOMS OF THE "STREAK" DISEASE

*On Sweet Pea.* Like the Bacteriosis of beans, it makes its appearance in the season of heavy dew. On the sweet pea the disease usually appears just as the plants begin to blossom; it is manifested by light reddish brown to dark brown spots and streaks (the older almost purple) along the stems, having its origin usually near the ground, indicating distribution by spattering rain and infection through the stomata (See Pl. 3). The lesions which at first are separate and distinct soon confluent meet, causing the streaked appearance. The disease becomes quickly distributed over the more mature stems until the cambium and deeper tissues are destroyed in continuous areas when the plant prematurely dies. Occasionally petioles and leaves show infection, the latter show the watersoaked spots common to the bacterial leaf blight of beans.

The disease is not a vascular infection; it confines its attack to the mesophyll, the cambium and deeper parenchymous tissues; the lesions on the stems gradually enlarge and deepen till they come together. On clovers (*Trifolium* spp.) the disease first appears in August and September on the young seedling plants, when often it is particularly severe, vying with Bain's Anthracnose<sub>20</sub> in its activity. In young clover it causes leaf spot (See Plates 7-12), water soaked as in the bean, and it also attacks the petioles and crown. In more mature clover probably the most severe attack takes place in the petiole and sheath at the union with the stem; in this case the entire leaf dies and the lesion extends down into the stem. The blackening of the stems and the spotting and water soaking of areas on the leaves are common with the clovers (See Plates 6, 7, & 8). It is not uncommon to find the blackened lesions on the stems of clover so overlapped as to cause the entire stem to darken.

*Soy Bean.* The disease was particularly severe upon one variety of soy bean, in which the lower lesions girdled the stem or penetrated so deeply that the plants blew over, suggesting the black leg of potatoes (See Plates 13, 14, and 15). The lesions were common on the



upper branches and even the pods, the latter showing very conspicuous blackened spots (Plate 14).

*Bean* (*Phaseolus* spp.) On several varieties of beans a stem lesion, which was supposed to be caused by *Pseudomonas phaseoli*, showed upon culturing an organism similar to that met with in the sweet peas, the clovers, and the soy beans. These lesions usually were small, elongated, rusty brown areas one-fourth to several inches in length; occasionally the lesion was slightly sunken (Pl. IV).

In order to compare this organism with that of *Pseudomonas phaseoli* of bean blight some 350 isolation plates were made from beginning lesions on bean stem and leaves. Instead of *Ps. phaseoli* in the stem lesions, in most cases this new organism was found. From water-soaked pods and leaves a *Pseudomonas* was obtained which answered closely to *Ps. phaseoli* (Pl. 5).

In order to determine the position of our work, extensive isolations, cross infections, and systematic studies were started.

### ISOLATION AND MORPHOLOGICAL STUDIES

Over 1500 plate cultures of beginning or young lesions were made from the several hosts. The organism may be taken almost invariably in abundance in pure culture from the beginning lesions in the stems of sweet peas when the surface is properly sterilized. Some difficulty was experienced at first in taking the organism from the clovers. This we attributed to non selection of young lesions, to too severe surface sterilization and to the ease with which the lesions in the thin cambium of the clover dry out, thereby causing the death of the organism.

The isolation work clearly indicated that the parasite was bacterial; a yellow organism which grows luxuriantly upon all the nutrient media and especially rapid upon nutrient media containing sugars. On standard nutrient glucose agar the colonies appear within 24 to 36 hours. The center becomes granular and the colonies have a marked tendency to become stellate or auriculate.

Morphological studies showed the organism to be a comparatively small rod-shaped bacillus, which in fresh cultures is never found in chains, and seldom even united in twos or fours. The flagella are not easily demonstrated. (See Fig. 1).

## INOCULATION EXPERIMENTS

Preliminary inoculation work was carried out Aug. 9, 1912 with sweet peas using two cultures, viz. sources No. 1 and No. 3 (see p. 17). The former came from sick sweet peas at the Experiment Station farm at Newark, Del., and the latter from sweet peas affected with "streak" from Boston, Mass. Inoculations were carried out by atomizer sprayings with 48 hour cultures, after having first thoroughly wet the plants. The sprayings were applied in the evening on all parts of the plants above ground. Typical "streak" infections showed on the 9th day. On the 10th day lesions were cut out, surfaces sterilized, washed, crushed, and cultured with the result that the typical organism used in the inoculation work was recovered. Inoculations were repeated on Aug. 22, 1912, this time using cultures of source No. 8 from sweet peas affected with "streak" sent here by T. A. Weston from Orpington, England, and source No. 13a from Red Clover. The inoculations were this time both sprayed on certain plants with atomizer, while others were pricked with hypodermic needle. In both cases, infection was obtained in from 7 to 10 days. The spots pricked by the needle were the first to show infection, these appearing in seven days. The red clover cultures gave equally strong infections as did the sweet pea. Subsequent platings of these infections gave an organism identical with that used in the inoculation work.

Several of the sources used in the cultural and biological studies were those secured from this preliminary infection work, viz. cultures No. 4, No. 5, and No. 7 and No. 10 E.

Following the above preliminary inoculation experiments a series of cross infection work (Sept. 5) was tried out in the field with only partial success. Tall moist chambers were placed over small young plants of clover, alfalfa, cowpeas and soybeans, using cultures from sweet pea and from red clover. The red clover showed infections from the bacteria taken from *Lathyrus* spp. while, on the other hand the cowpea, soy bean and alfalfa showed no infection whatever. On Sept. 15, this series was again tried out with only evidence of infection on the red clover. It would seem that alfalfa, and cowpea as well as soy bean in the young stages are not readily susceptible to this parasite.

## CROSS INOCULATION IN THE LABORATORY

About the first of October (1912) some two dozen red clover plants were transplanted in eight inch pots and taken into the laboratory where they made excellent growth. All evidence of infection was

removed and the leaves were thoroughly sprayed with potassium permanganate to surface sterilize them; the disinfectant was later carefully washed off; the clover plants were then placed under moist jars for several days to note whether any natural infection would follow. The sterile plants were then thoroughly covered by atomizer, first with sterile water and then sprayed with young cultures of the streak disease bacterium from ten different sources, viz., one from red clover and one from alsike clover, two from sweet pea, one from another *Lathyrus* sp., two from soy bean, one from Lima bean, one from alfalfa, and one from infected soil.

The following table shows the amount of infection on the clover leaves and stems.

*Table Showing Amount of Artificial Infection on Red Clover*

1. Red Clover on Red Clover	10% of infection
2. Sweet Pea (American) on Red Clover	12% " "
3. <i>Lathyrus</i> sp. " " "	99% " "
4. Soy Bean " " "	4% " "
5. Soy Bean " " "	60% " "
6. Sweet Pea (English) " " "	50% " "
7. Alsike " " "	75% " "
8. Lima Bean " " "	40% " "
9. Alfalfa " " "	35% " "
10. Soil " " "	90% " "

The results of these infections are shown in Plates 10, 11 and 12. Infection began in 3 to 7 days. The leaf lesion is a typical water-soaked area, quite similar to that of the bean blight disease.

These experiments were carried out again beginning Nov. 11, 1912, along with some additional inoculations. *Pseudomonas phaseoli* of bean blight was tried on red clover but it failed to produce any infection. Three sources of the "streak" disease organisms were used from sweet pea, and two sources from forage species of *Lathyrus*. Infection was evident in three days. Two sources of the "streak" organism *B. lathyri* from beans were used getting definite infection in three to five days. From this work it was quite apparent that the organism from the several different hosts was capable of producing the disease on red clover identical with that in the field.

Many different attempts were made to get infection by spraying on clover and sweet peas and not paying any attention to keeping the

plants moist. Invariably the results were negative. All attempts to infect young sweet pea plants have been failures. The disease will not develop till the plant is about to blossom. Just what physiological changes take place in the plant just prior to and at the time of blossoming has not been determined, but it is evident that there must be a radical change, as needle inoculations or sprayings will make no advance whatever.

The writer is of the opinion that along with a possible change in the physiology of the plant, owing to the fact that the flowering period falls in the time of heavy dew, there is a turgescence in the plant throughout the nights which probably favors the entrance and growth of the organism into the interior of the plant.

### THE RELATIONSHIP OF THE CASUAL ORGANISMS

The series of apparently similar organisms taken from such a variety of hosts offered a splendid opportunity for studying the possible cultural and biochemical variation. Having proved that red clover could be infected with the organisms from the different sources (see p. 14) these same sources, together with several morphologically similar organisms from other hosts were used for a detailed study of variation in cultural, physical and bio-chemical features. In order to be certain that the media were standard in their properties and reactions, several well known organisms were run as checks. For this purpose *Bacillus coli*, *Pseudomonas compestris* and *Pseudomonas phaseoli* were selected. The cultures used in this study were taken from beginning lesions which appeared free from contamination.

The isolation of bacteria from lesions was carried out by following a method which has been used by us<sub>21</sub> for a number of years.

This consists in cutting out the lesions somewhat beyond the area of infection, pieces being small enough to be placed in a culture tube. If the lesions are on small stems, as that of clover or sweet pea, sections of the stem, including lesions are cut out one-half to three-fourths of an inch long. If the lesions are on the leaf, the youngest infections are selected and taken out entire. The surface sterilization is carried out in a clean test tube, upon several pieces at a time, using enough of a 50% alcoholic solution containing one gram of bichloride of mercury to the liter, to cover the infected pieces of plant tissue at a depth of one inch. After placing the plug in the tube and shaking the disinfectant to all parts of it, the solution is allowed to stand on the material from 15 seconds to two minutes, depending on the nature of the plant tis-

sue. Thin leaves, lightly infected, will hardly stand more than 10 seconds, while deeply infected stems and tubers, or root tissues will stand two minutes, or even much more when the disinfectant does not penetrate too deep. At the required time, the disinfectant is poured off and the material is washed three times with 12 to 15 cc. of sterile water for each washing. This washing is carried out in the same tube in which the surface sterilization is done. Sterilized water for this purpose is kept in stock at all times in the laboratory, stored in tubes containing 12 to 15 cc. After flaming the plug and mouth of the tube carrying sterile water, the contents is poured directly upon the material to be washed and the flamed plug is used to close the tube. The water is thoroughly shaken to all parts of the tube in order to wash away every trace of the disinfectant. This water is then poured off and the process repeated, each time using the new cotton plug from the sterile water tube, until three washings have been given, when the material is gathered near the mouth of the tube. Here the individual pieces are picked out with sterile forceps and each is crushed with the same forceps in the mouth of a tube containing a medium properly cooled for growth. The crushed lesion is washed down by the medium, sub-cultures are made and poured into sterile plates.

The writer has had excellent success with this method, succeeding almost invariably in taking out pure cultures of the pathogen, whenever it is not associated deeply in the lesion with contaminating organisms.

The following is a list of the organisms isolated and used in the cultural studies:

- No. 1. From Sweet Pea Stem, showing typical "streak" obtained July 20, 1912 from the Experiment Station farm at Newark, Del.
- No. 2. From Sweet Pea Stem showing typical "streak" obtained July 25, 1912 from the Experiment Station farm at Newark, Del.
- No. 3. From Sweet Pea Stem showing typical "streak" obtained Aug. 1, 1912 from the noted sweet pea grower, Mr. William Sim of Boston, Mass.
- No. 4. From Sweet Pea Stem showing typical "streak," artificial infection in garden of T. F. Manns, Newark, Del., Aug. 18, 1912.

- No. 5. From Sweet Pea Stem showing typical "streak" by artificial infection in garden of T. F. Manns, Newark, Del., Aug. 18, 1912.
- No. 7. From Sweet Pea Stem showing typical "streak" by artificial infection in garden of T. F. Manns, Newark, Del., Aug. 21, 1912.
- No. 8. From Sweet Pea Stem showing typical "streak" obtained Aug. 22, 1912. from T. A. Weston, St. Johns Road, Orpington, England.
- No. 9. From Sweet Pea Stem showing typical "streak" obtained Aug. 24, 1912 from T. A. Weston, St. Johns Road, Orpington, Eng.
- No. 10 e. From Sweet Pea Stem showing typical "streak" from artificial infection Aug. 22, 1912. from T. F. Manns' garden, Newark, Del.
- No. 12 d' From Sweet Pea Stem showing typical "streak" obtained from J. J. Taubenhaus' garden, Newark, Del., Sept. 24, 1912.
- No. 12. h'. From Sweet Pea Stem showing typical "streak" obtained from J. J. Taubenhaus' garden, Newark, Del., Sept. 24, 1912.
- No. 43 c. From Sweet Pea Stem, showing typical "streak" obtained from Marshall Manns' Garden, Oct. 25, 1912, Newark, Del.
- No. 13 a. From Red Clover petiole, showing black lesion in cambium, obtained Sept. 4, 1912 from the Experiment Station Farm, Newark, Del.
- No. 14 i'. From Red Clover Stem, showing darkened lesion in cambium, obtained Sept. 7, 1912 in vicinity of Newark, Del.
- No. 15. From Red Clover Stem, showing black lesion in cambium, from field near Mr. Taubenhaus' home, Newark, Del., Sept. 24th, 1912.
- No. 16 i. Same source as No. 15. Different plant.
- No. 41 K. From Red Clover Stem showing black lesion in cambium, called "OK Culture" because material so typical, from vicinity of Newark, Del., Sept. 25, 1912.

- No. 17 d'. From Alsike Clover petiole, showing typical blackened lesion, from field near Red Men's Home, Newark, Del., Sept. 4, 1912.
- No. 18 e'. From Alsike Clover petiole, same origin and date as No. 17 d' though different plant.
- No. 21. From Soy Bean petiole, showing small sunken black lesion, from Experiment Station farm, Newark, Del., Sept. 3, 1912.
- No. 22. From Soy Bean Stem, showing beginning black lesion, from Experiment Station farm, Newark, Del., Sept. 7, 1912.
- No. 23 h'. From Soy Bean Stem, showing beginning lesion, from Experiment Station farm, Newark, Del., Sept. 24, 1912.
- No. 24 h'. From Soy Bean stem, from beginning black lesion, same source and date as No. 23 h, though different plant.
- No. 25 d<sub>2</sub>. From Lathyrus Sp. (for forage purposes) showing typical "streak" lesions on stem, obtained from Experiment Station farm, Newark, Del., Sept. 3, 1912.
- No. 26 e'. From Lathyrus Sp. (for forage purposes) showing typical "streak" lesions on stem, obtained from Experiment Station farm, Newark, Del., Sept. 27, 1912.
- No. 26 f'. From Lathyrus Sp. Same source and date as No. 26 e' only a different plant.
- No. 28. From Cowpea Leaf, sent in from vicinity of Philadelphia, leaves turning brown: Aug. 3, 1912.
- No. 30. From Wax Bean seed taken from green pod showing large watersoaked lesion; obtained from Red Men's Home, Newark, Del., Sept. 3, 1912.
- No. 36. From Tomato fruit showing typical end rot of fruit, from T. F. Manns' garden, Newark, Del., July 13, 1912.
- No. 37. From Tomato fruit end rot produced artificially by inoculation with source 26 above, from infection in laboratory, Newark, Del. Variety of Tomato "Stone," July 28, 1912.
- No. 40 g<sub>2</sub>. From Climbing Bean pod (green string bean). Typical brown sunken lesions along pod; from Mr. Taubenhaus' garden, Newark, Del., Sept. 30, 1912.

- No. 42. From Dwarf Bean, watersoaked pod, plate No. 15, from Mr. Taubenhause's garden, Newark, Del., Oct. 27, 1912. This organism is *Ps. phaseoli* and was hence used as a check.
- No. 45. From Dwarf Bean Pod. Dark brown lesions along pod; from Mr. Taubenhause's garden, Newark, Del., Oct. 27, 1912. This organism is also *Ps. phaseoli*.
- No. 46. From Dwarf Bean Pod. Typically watersoaked lesion from Mr. Taubenhause's garden, Newark, Del., Oct. 27, 1912. This organism is also *Ps. phaseoli* and used as a check.
- No. 47. *Bacillus coli* from human intestine. Used here as a standard check on media and for reactions.

## CULTURAL AND BIOCHEMICAL STUDIES

Extensive cultural and biochemical studies made upon the thirty-two different strains from the several hosts confirm the results from inoculation work, viz. that the causal organisms are identical as far as our present system of classification is concerned. Some slight variations were noticeable in several of the strains, such as absence of pellicle in nutrient broth, or a very meagre pellicle. These differences were just as marked in the different strains from the one host as they were between the strains from the several hosts. This was likewise true for color gradations on the different solid media. The shades of yellow varied from a light straw to that of almost a deep orange. Probably the most uniform cultural reaction was that on nutrient gelatin, in which in practically every strain it required somewhat over two months for complete liquifaction, while at the end of six weeks not more than half of the gelatin was liquified. The fact that liquifaction proceeded entirely from above would indicate that the organism is an obligate aerobe.

In table I is shown in a brief summary the group number and other features of the thirty-seven organisms compared thruout the cultural studies. It is quite apparent that of the 32 strains first considered in this summary and selected from various hosts because of similarity in disease production, or because of similarity in morphological and preliminary cultural studies, that there is not variation enough to differentiate one strain from another on any specific cultural reaction (See Table I).



The introduction into this series of cultures for comparative studies of sources Nos. 36 and 37 from "point" or "fruit rot" of tomato was to learn the cultural and other classificatory features of the organism which here is shown to be the active causal agent of point rot. (see Pl. 16). As far as the present system of bacterial classification is applicable, this organism is not to be distinguished from that described herein as *Bacillus lathyri* n. sp. The writer did not carry out cross inoculation work on sweet pea and clover with the organism from the point rot of tomato. Though the morphological and cultural features of the tomato organism are similar as far as we are able to determine by present methods, yet it is quite possible that the organism may not be able to cause the "streak" disease on sweet pea and other legumes.

A short history of the association of bacteria with the "fruit rot" or "blossom end rot" of tomato is as follows:

Elizabeth H. Smith<sub>22</sub> (1905) and F. S. Earle<sub>43</sub> (1900) have found bacteria associated with the fruit rot of the tomato. Both claim typical infections when inoculations were made from pure isolated cultures. William A. Stuart<sub>24</sub> has also found bacteria associated with the fruit rot of the tomato. The writer in July 1912 found the rot quite general in his garden, and having seen Miss Smith's report of a "bacterial rot" of the tomato took the opportunity of culturing young lesions from affected fruit. Careful surface sterilization was practiced; only beginning lesions were cultured in the first series and the extreme margins of the lesions were used. The result was that in every plate a yellow bacterium came out. In several of the plates there were associated occasionally a *Fusarium* and an *Alternaria*. Both of these fungi as well as the bacterium were isolated in pure culture on an artificial medium. Inoculation work was carried out with each of the organisms. The bacterium gave excellent infection both in the field and in the laboratory during a period of dry weather, when sprayed with atomizer or inoculated by needle prick. During the same time there was also some evidence of infection when the *Fusarium* was inoculated into the flower end of the fruit by inserting mycelium beneath the epidermis with a sterile scalpel. The *Alternaria*, however, made no progress at all. The check sprayings with sterile water and the check incisions gave no infections. The above experiments were duplicated during a period of rainy, cloudy weather with very little progress whatever in the production of rot. The *Fu-*

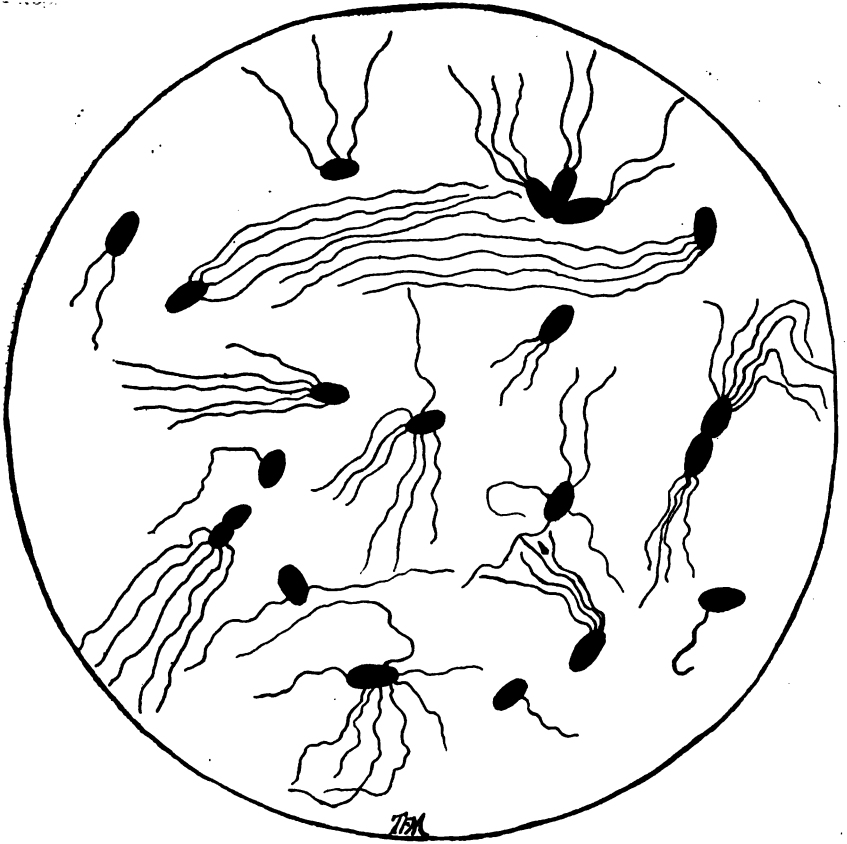


Fig. 1

sarium and *Alternaria* showed no evidence of infection, while the bacterium produced only small watery lesions.

Drought is a great factor in furthering the disease. Heavy rains with moist atmosphere entirely checked the disease in the experimental work. The writer is inclined to infer that this disease is of bacterial origin and that its progress is very closely associated with the water supply of the plant.

(Since writing the above in June 1913 the writer has been privileged to review Brooks' work on the "Blossom-end Rot of Tomatoes," *Phytopathology* Vol. VI, No. 5, Oct. 1914. He still holds the view that the disease is of bacterial origin, and is distributed by rain or insects at blossoming time. The very beginning lesions give pure cultures of the bacterium.)

## DESCRIPTION OF THE CAUSAL ORGANISM\*

*Bacillus lathyri* n. spp. Manns & Taubenhauß

### I. MORPHOLOGY

1. *Vegetative Cells.* When grown upon nutrient agar for 24 hours at 25° to 28° C. and stained with aqueous solutions of methylene blue, gentian violet or fuchsin the organism is shown to be a comparatively small rod-shaped bacillus, with rounded ends which is rarely found in two or fours. The stain in the organism from such cultures is evenly distributed thruout the cytoplasm. In older cultures, two to three months, some take a denser polar stain. The organism measures from .75<sup>u</sup> to 1.5<sup>u</sup> X .6<sup>u</sup> to .85<sup>u</sup> the majority being 1.4<sup>u</sup> X .75<sup>u</sup>.

2. *Sporangia.* No sporangia have been observed.

3. *Endospores.* Cultures on various media carried for ten months show no endospores.

4. *Flagella.* When stained by Loeffler's, Pitfield's or Van Ermengen's methods the flagella may be demonstrated, though not easily. They are shed so readily that usually no more than from two to five may be shown attached, though it is not uncommon to find many detached thruout the field. However, when the material is carefully selected, fixed and stained, the flagella may be demonstrated to be well distributed peritrichially, and to number eight or even more. (See Fig. 1).

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\*The thirty-seven descriptive charts used in tabulating the morphological, cultural, physical and biochemical features of the different organisms, are not printed herewith.



† Star(\*) signifies liquefaction very slow, requiring three months  
 \* Fluorescent in Asparagin Broth. ‡ Note destruction of starch very slow

†† Start(\*) signifies liquidation very slow, requiring three months

\* Fluorescent in Asparagin Broth.

In hanging drop or under cover slip the organism in 24 to 48 hour cultures is very active, taking a motion somewhat slower but otherwise similar to that of *B. typhosus*.

5. *Capsules*. No capsules have been demonstrated.

6. *Zoogloea*. No zoogloea has been formed on the ordinary media, but in asparagin broth, and in Uschinsky's solution a ropiness is common; on starch jelly containing Uschinsky's solution a pseudozoogloea is present.

7. *Involution forms*. Individuals somewhat longer and broader than usual are met with in old cultures four to six months; bipolar staining, and denser regions of cytoplasm are commonly seen with ordinary stains. Some individuals show granulation, though this is not common.

8. *Staining reactions*. The organism, though staining readily with the ordinary stains, loses these quickly when washed with alcohol.

9. *Gram's Stain*. The organism is gram negative.

10. *Loeffler's Methylene Blue*. With Loeffler's Methylene blue the cytoplasm shows no granulation and is evenly stained thruout from a 24 hour culture.

11. *Neisser's Spore Stain*. No evidence of spores present with Neissers' stain.

## II. CULTURAL FEATURES

### 1. *Agar Stroke—Nutrient Agar*.

*Growth*, at 25 to 28° rapid in 24 hours, and abundant in 72 hours. No growth at 37° C.

*Form of Growth*, filiform, usually smooth at margin but occasionally undulate, or even slightly echinulate.

*Elevation of Growth*, slightly convex.

*Luster*, glistening.

*Topography*, usually smooth; surface occasionally very slightly granulate.

*Optical Characters*, opaque.

*Chromogenesis*, light to deeper yellow in different strains.

*Odor*, absent.

*Consistency*, butyrous.

*Sub medium*, shows no change of color.

## 2. *Potato*

*Growth*, quite rapid in 48 hours.

*Form of Growth*, filiform becoming more or less irregular.

*Elevation of Growth*, slightly convex.

*Luster*, glistening.

*Topography*, smooth.

*Chromogenesis*, at first a light yellow, later becoming somewhat deeper.

*Odor*, absent.

*Consistency*, butyrous and slightly viscid.

*Medium*, not changed.

## 3. *Loeffler's Blood Serum*.

(not used)

## 4. *Agar Stab*.

*Growth*, best at top-surface growth quite rapid in 48 hours, abundant after 3 days. Organism a rather closely restricted aerobe.

*Line of Puncture*, filiform, but quite restricted, in growth deep in the medium.

*Chromogenesis*, yellow.

*Medium*, not changed in color.

## 5. *Gelatin Stab*,

*Growth*, best at top, small amount of growth in the lower part of stab.

*Line of Puncture*, filiform.

*Liquifaction*, slow, not showing till nearly two weeks old and at end of four weeks only fairly well begun. Not complete till three months. Quite uniform thruout 32 strains.

*Medium*, not discolored.

## 6. *Nutrient Broth*.

*Surface Growth*, slight pellicle in some cases though not general.

*Clouding*, strong in 24 hours.

*Odor*, absent.

*Sediment*, compact, scant.

7. *Plain milk*... No visible change takes place in two weeks. At the end of three weeks 23 out of the 32 strains showed curdling without separation of whey or of digestion of curd, while 9 strains did not show coagulation until heat was applied. Tests showed that the acidity in the various strains which coagulated milk in three weeks gradually increased from 0 Fuller's scale to +6 at end of the third day; to +12 at end of first week, and at time of coagulation, that is three weeks, had reached +36, Fuller's scale. It seems probable that where coagulation took place before evidence of peptonization of casein set in, that the increased acidity may have in some strains checked the growth of the organism. In the 9 strains noted above some evidence of digestion as well as acid production was showing though the acidity was not great enough to bring about coagulation. At the end of seven weeks 24 strains showed partial or complete digestion of casein, while eight remained curded with little or no evidence of digestion. In several strains there was evidence of curdling from enzymatic action; that is, the production of acid did not appear strong enough to bring about coagulation.

8. *Litmus Milk*.

In most strains there was a gradual and slow increase in acidity thruout the first month. In several of the more rapid digesters of casein the litmus was also digested and reduced.

9. *Gelatin Colonies*. Eight days old.

*Growth*, medium.

*Form*, round.

*Elevation*, slightly convex.

*Edge*, smooth.

*Liquifaction*, too slow to show on plate.

10. *Agar Colonies*, five days old.

*Growth*, rapid at 23 to 28° C.—Yellow colonies, visible to the eye in 24 hours.

*Form*, stellate to ameboid.

*Surface*, smooth, glistening.

*Elevation*, slightly raised.

*Edge*, entire and regular.

*Internal Structure*, granular at center and smooth elsewhere.



*Chromogenesis*, yellow.

*Size*, depends on room in plate, much extended.

11. *Glycerine agar.*

*Growth*, rapid and abundant at 23 to 28° C.

*Form of Growth*, filiform.

*Elevation of Growth*, slightly raised.

*Luster*, glistening.

*Topography*, smooth.

*Optical characters*, opaque.

*Chromogenesis*, light yellow.

*Odor*, none, or slightly sour.

*Medium*, not changed in appearance.

12. *Synthetic agar low in nitrogen.*

Weak to no growth.

13. *Cohn's solution* at 23 to 28° C.

*Growth*, absent.

14. *Uschinsky's solution* at 23 to 28° C.

*Growth*, rapid in 24 hours.

*Fluid*, viscid yellow sediment after three days; sometimes a pellicle would form and sink, followed by others. No fluorescence.

15. *Dunham's solution* at 23 to 28° C.

*Growth*, moderate.

*Clouding*, moderate, persistent, fluid, slightly turbid.

Indol formed.

16. *Asparagin solution* at 23 to 28° C.

*Growth*, abundant.

*Clouding*, moderate, persistent, fluid turbid.

17. *Nitrate Broth* at 23 to 28° C.

*Growth*, moderate.

*Clouding*, moderate, persistent, fluid slightly turbid.

*Nitrates*, not reduced.

18. *Dextrose Bouillon* at 23 to 28° C.

*Growth*, abundant.

*Clouding*, strong, persistent, fluid turbid. Gradually becomes acid from +7 Fuller's scale to +14 in 7 days.

19. *Saccharose Bouillon* at 23 to 28° C.*Growth*, abundant.*Clouding*, strong, persistent, fluid turbid. Gradually increases in acidity from +3 Fuller's scale to +9 in 7 days.20. *Lactose Bouillon* at 23 to 28° C.*Growth*, abundant.*Clouding*, strong, persistent, fluid turbid. Increases in acidity.21. *Maltose Bouillon*.*Growth*, abundant.*Clouding*, strong, persistent, fluid turbid. Increases in acidity.22. *Glycerine Bouillon* at 23 to 28° C.*Growth*, abundant.*Clouding*, strong, persistent, fluid turbid. Increases in acidity.23. *Mannite Bouillon* at 23 to 28° C.*Growth*, abundant.*Clouding*, strong, persistent, fluid turbid. Increases in acidity.24. *Growth on Bouillon over Chloroform*.*Growth*, absent.25. *Sodium Chloride in Bouillon*.

4% inhibited growth.

TABLE II-A.

2. *Production of Acid and Alkali* (Fullers Scale)

Sugar Free Broth plus 1%	Degrees of Reaction after			
	0 days	3 days	5th day	7th day
Dextrose	+7	+10	+12	+14
Saccharose	+3	+ 6	+ 8	+ 9
Lactose	+6	—	—	Increased
Maltose	+8	—	—	"
Glycerine	+2	—	—	"
Mannite	+6	—	—	"

TABLE II-B. Showing Production of Acid\* in Dextrose and Saccharose Broth in seven days.

Number of Culture and Host		Am't of 10 Acid in 100cc of Dextrose Broth	Am't of 10 Acid in 100cc of Saccharose Broth	Number of Culture and Host		Am't of 10 Acid in 100cc of Dextrose Broth	Am't of 10 Acid in 100cc of Saccharose Broth
1	Sweet Pea	4.5 "	0.0 "	21	Soy Bean	6.0 "	9.0 "
2	"	8.5 "	6.3 "	22	"	7.5 "	7.0 "
3	"	3.0 "	7.0 "	23h	"	7.5 "	10.0 "
4	"	4.5 "	7.3 "	24h	"	5.0 "	8.0 "
5	"	5.5 "	7.0 "	25d <sub>2</sub>	Lathyrus spp.	7.0 "	8.3 "
7f	"	3.0 "	5.6 "	26e	"	6.5 "	7.0 "
8	"	6.5 "	5.6 "	26f	"	9.0 "	9.0 "
9d	"	7.0 "	7.6 "	28	Cow Pea	5.5 "	5.3 "
10e	"	4.5 "	8.3 "	30	Wax Bean Pod	4.5 "	10.0 "
12d <sup>a</sup>	"	8.0 "	5.6 "	36	Tomato End Rot	7.5 "	5.3 "
12h <sup>a</sup>	"	10.0 "	7.6 "	37	"	7.0 "	10.3 "
43c	"	9.5 "	6.6 "	40g <sub>2</sub>	Climbing Bean Pod	7.5 "	8.3 "
13 <sup>a</sup>	Red Clover	5.5 "	7.6 "	42	Dwarf Bean Watersoaked	1.5 "	1.0 "
14j	"	6.0 "	7.0 "	44	Cabbage, Black Rot	9.0 "	1.0 "
15	"	4.5 "	5.6 "	45	Dwarf Bean Pod	.0 "	1.3 "
16c	"	5.0 "	9.6 "	46	Dwarf Bean Pod	2.0 "	.0 "
16i	"	5.0 "	9.0 "	47	Bacillus coli	9.0 "	.3 "
41h	"	6.5 "	7.6 "				
17	Alsike Clover	9.0 "	5.6 "				
18	"	6.5 "	6.3 "				

\* The acidity of the check has been deducted from the total  
thruout these experiments.

26. *Nitrogen*, apparently is not obtained from the atmosphere, but is obtained from all the broths.

27. *Best medium for long continued growth.*

Nutrient glucose agar has given the longest growth in a shake deep tube culture.

### III. PHYSICAL AND BIOCHEMICAL FEATURES.

1. *Gas production.* No gas is produced from dextrose, saccharose, lactose, maltose, glycerine and mannite broth in fermentation tubes, very little or no growth takes place in the arm. The limitation of growth was sharply defined at the union of bulb and arm, indicating an obligate aerobe.

3. *Production of Ammonia.* The production of ammonia has been determined in nutrient broth, Dunham's peptone solution, asparagin solution and nitrate broth. In all of these solutions some ammonia was present. The ammonification was quantitatively measured in a 1% peptone solution. The following table (III) shows the amount of N/10 ammonia formed in 100 cc. of above peptone solution in seven days and twelve days respectively by eight different strains of *Bacillus lathyrus* from the following hosts;—sweet pea (3), Red Clover (2), alsike clover (1), soy bean (1), and wax bean (1).

TABLE III  
Showing Ammonification

No. of Culture and Host	In Seven Days			In Twelve Days		
	Total $\frac{N}{10}$ $\text{NH}_3$	$\frac{N}{10}$ $\text{NH}_3$ in check	$\frac{N}{10}$ $\text{NH}_3$ produced	Total $\frac{N}{10}$ $\text{NH}_3$	$\frac{N}{10}$ $\text{NH}_3$ in check	$\frac{N}{10}$ $\text{NH}_3$ produced
43 C. Sweet Pea	9.1 cc	1.6 cc	7.5 cc	14.6 cc	1.9 cc	17.7 cc
9 d <sub>2</sub> " "	7.2 "	1.6 "	5.6 "	13.2 "	1.9 "	11.3 "
10 e " "	7.2 "	1.6 "	5.6 "	13.2 "	1.9 "	11.3 "
13a Red Clover	6.7 "	1.6 "	5.1 "	11.5 "	1.9 "	9.6 "
14 i " "	3.9 "	1.6 "	2.3 "	10.9 "	1.9 "	9.0 "
18 Alsike Clover	6.0 "	1.6 "	4.4 "	14.2 "	1.9 "	12.3 "
21 Soy Bean	7.9 "	1.6 "	6.0 "	13.8 "	1.9 "	11.8 "
40g <sub>2</sub> Wax Bean	6.1 "	1.6 "	4.5 "	12.4 "	1.9 "	10.5 "
Soil inoculation	58.1 "	1.6 "	56.5 "	74.8 "	1.9 "	72.9 "
Check Culture	1.6 "	1.6 "	1.0 "	1.9 "	1.9 "	0.0 "

4. *Nitrates in Nitrate Broth.* No nitrate reduction takes place. Several of the strains produce nitrites from peptone broth; thus in nitrate broth unless quantitative determination is made for the nitrates

placed therein one might easily assume nitrate reduction. Ammonia is not formed from nitrates.

TABLE IV

*Showing Growth on Acid and Alkali Media.*

Culture and Host		-10	0	+15	+25	+35
1	B. lathyri, Sweet pea	+	+	+	0	0
2	" " " "	+	+	+	0	0
3	" " " "	+	+	+	0	0
4	" " " "	+	+	+	0	0
5	" " " "	+	+	+	0	0
7f	" " " "	+	+	+	0	0
8	" " " "	+	+	+	0	0
9d <sub>2</sub>	" " " "	+	+	+	0	0
10e	" " " "	+	+	+	0	0
12d'	" " " "	+	+	+	0	0
12h'	" " " "	+	+	+	0	0
43c	" " " "	+	+	+	0	0
13a	" " Red Clover	+	+	+	0	0
14i	" " " "	+	+	+	0	0
16c	" " " "	+	+	+	0	0
15	" " " "	+	+	+	0	0
16c	" " " "	+	+	+	0	0
16i	" " " "	+	+	+	0	0
41k	" " " "	+	+	+	0	0
17	" " Alsike Clover	+	+	+	0	0
18	" " " "	+	+	+	0	0
21	" " Soy Bean	+	+	+	0	0
22	" " " "	+	+	+	0	0
23h'	" " " "	+	+	+	0	0
24h'	" " " "	+	+	+	0	0
25d <sub>2</sub>	" " Lathyrus spp.	+	+	+	0	0
26c	" " " "	+	+	+	0	0
26f	" " " "	+	+	+	0	0
28	" " Cow pea	+	+	+	0	0
30	" " Wax Bean pod	+	+	+	0	0
36	" " Tomato End Rot	+	+	+	0	0
37	" " " " "	+	+	+	0	0
40g <sub>2</sub>	" " Climbing Bean Pod	+	+	+	0	0
42	Ps. phaseoli Dwarf Bean Pod	+	+	+	0	0
44	Ps. campestris Black Rot Cabbage	+	+	+	0	0
45	Ps. phaseoli, Dwarf Bean Pod	+	+	+	0	0
46	Ps. phaseoli, Dwarf Bean Pod	+	+	+	0	0
47	Bacillus coli.	weak	+	+	0	0

5. *Indol Production.* Indol was produced in each of the 32 strains tested out. In several the reaction for indol was light while

in the majority it was nearly equal to that in the check culture, viz. *Bacillus coli*.

6. *Relation to acid and alkali.* The 32 strains of *Bacillus lathyri* together with 3 strains of *Ps. phaseoli*, one strain of *Ps. campestris* and one strain of *Bacillus coli* were tested on a broth medium varying in acidity and alkalinity. Table IV shows growth (+) and absence of growth (—) on media of following reactions —10, 0, +15, +25, and +35 Fuller's scale.

7. *Optimum Reaction.* The optimum reaction is between 0 and +10 Fuller's Scale.

8. *Vitality on Culture Media.* Vitality on culture media is quite prolonged. Eight months' old shake cultures on deep, moist nutrient glucose agar gave strong growth when transferred to plate dilutions and to slant cultures.

9. *Temperature Relations.*—*Thermal Death Point*, determined by heating tubes containing 10 cc. of broth to the desired temperature, then inoculating with loops of fresh culture in bouillon, allowing the inoculated tube to remain in water at same temperature for 10 minutes, then removing and cooling. Cloudiness within ten days indicated growth. The thermal death point is between 48° C and 50° C.

The following table shows the thermal death point for five sources of *B. lathyri* and three sources of the checks viz. *Ps. phaseoli*, *Ps. Campestris* and *B. coli*. Each was run in duplicate and incubated 10 days. Where the sign plus and minus is used it indicates that one of the tubes showed growth while the others did not.

TABLE V. THERMAL DEATH POINT

(Incubated 10 days)

			46°C	48°C	50°C	52°C	54°C
8	<i>B. lathyri</i>	Sweet Pea—England	+	+	—	—	—
43 C	"	" " —Delaware	+	+	—	—	—
41 K	"	Red Clover "	+	+	—	—	—
40g <sub>2</sub>	"	Pean "	+	—	—	—	—
22	"	Soy Bean "	+	+	—	—	—
42	<i>Ps. phaseoli</i>	Bean "	+	+	—	—	—
44	<i>Ps. campestris</i>	Cabbage "	+	—	—	—	—
47	<i>B. coli</i>	Human Intestine, Pa.	+	+	+	+	—

*Optimum Temperature.* The optimum temperature lies between 28° to 30° centigrade.

At 37½° C. no growth occurs on any of the nutrient broths, or nutrient sugar broths. Lower temperatures than 20° C. have not been tried.

10. *Relation to Oxygen.* *Bacillus lathyri* n. sp. altho not strictly aerobic, makes so little growth in the absence of oxygen that it properly should be classed as an obligate aerobe. On stab cultures in different media, altho the line of stab is visible, yet the growth is very weak. In the arm of the fermentation tube little growth was manifested, and at the end of the 5th day the line of demarcation in growth between that in the bowl and that in the arm was very distinct.

11. *Production of Ferments.* The presence of a peptonizing enzyme is indicated by the liquifaction of gelatin; the time required, viz. two or three months for total liquefaction would indicate that this proteolytic enzyme is produced very sparingly.

Diastase is also produced which likewise is very weak in its action. The production of glucose cannot be demonstrated in a starch bouillon culture till the end of the second week. On potato plugs three months old, glucose may be shown to be present to a considerable extent, while check plugs of the same age showed no reduction of copper in Fehling's solution.

It is difficult to say whether rennet is present or not. In several cultures the coagulation in milk showed before there was an acidity strong enough (apparently) to be the cause of curdling. Whether the different acids produced vary in their coagulating power has not been determined.

*Oxidase.* If the production of gas in the presence of peroxide of hydrogen indicates oxidase then it is produced by each of the 32 strains.

*Lipase* is not present.

*Crystals* were not found present in any of the different media used.

## IV. PATHOGENICITY

Pathogenic for Sweet pea (*Lathyrus odoratus*) and other *Lathyrus* spp. for the clovers (*Trifolium* spp.) for soy beans (*Soja* spp.) and culinary beans (*Phaseolus* spp.)

## V. NUMERICAL CLASSIFICATION

According to the numerical system of recording the salient characters of an organism, *Bacillus lathyr* n. Sp. becomes B. 211. 2222522.

## MEDIA EMPLOYED

The ordinary media employed, viz. the nutrient broth, nutrient gelatin, nutrient agar, nutrient glucose agar and other sugar agars as well as sugar free broth have been prepared according to the recommendation for standard methods of Water Analysis. The reaction expressed in Fuller's scale was +10 except in certain special media in which cases the formulae of the originators or certain advantageous modification have been followed.

*Sugar Broths* were prepared by adding one percent of the different sugars as well as glycerine and mannite, to sugar-free broth.

*Glycerine agar* was prepared by adding four per cent of glycerine to nutrient agar.

The following are the formulae of the different media used.

*Dunham's Solution*

Distilled water .....	1000.0 cc.
Witte's Peptone .....	10.0 grams
NaCl .....	5.0 "

*Nitrate Broth*

Distilled water .....	1000.0 cc.
Witte's Peptone .....	1.02 grams
KNO <sub>3</sub> (nitrite free) .....	.2 "

*Asparagin Solution*

Distilled water .....	1000.0 cc.
Asparagin .....	2.0 grams
K <sub>2</sub> HPO <sub>4</sub> .....	1.0 "
MgSO <sub>4</sub> .....	1.0 "



*Uschinsky's Solution*

Distilled water .....	1000.0 cc.
Glycerine .....	40.0 grams
NaCl .....	5.0 "
CaCl <sub>2</sub> .....	0.1 "
MgSO <sub>4</sub> .....	.3 "
K <sub>2</sub> HPO <sub>4</sub> .....	2.0 "
Ammonium lactate .....	6.0 "
Sodium asparaginate .....	4.0 "

*Cohn's Solution*

Distilled water .....	1000.0 cc.
KH <sub>2</sub> PO <sub>4</sub> .....	5.0 grams
MgSO <sub>4</sub> .....	6.0 "
Ammonium tartrate .....	10.0 "
KCl .....	.5 "

*Synthetic Agar, Low in Nitrogen*

Distilled water .....	1000.0 cc.
Cane sugar .....	50.0 grams
KH <sub>2</sub> PO <sub>4</sub> .....	1.00 "
MgSO .....	.2 "
Shredded agar .....	15.0 "

*Litmus Milk.* Prepared by adding to plain milk one per cent of a solution of azolitmin made by dissolving 1 gram of azolitmin in 40 cc. of distilled water and kept at 37.5° C. for 12 to 18 hours.

The following media were used in a search for a quick differentiating medium; silicate jelly with Fermi's solution prepared according to Erwin F. Smith in his work, "Bacteria in Rel. to Plant Diseases." Vol. pp. 37-39; starch jelly containing Uschinsky's solution as modified by Smith. This medium gave exceptionally fine distinctions in coloring for the different species used as checks. *B. lathyri* gave a copious slimy yellow growth, varying in color from light straw to orange. The sub medium was not changed in color. *Ps. phaseoli* produced a very light yellow (almost colorless) slimy and copious growth; submedium slightly changed. *Ps. Campestris* produced a typical prune juice coloring similar to pneumonia sputum with an abundant growth; the submedium was also browned. *B. coli* colored the medium a typical light drab. The submedium was also slightly colored. We

consider this an excellent medium for differentiating purposes in general. The silicate jelly gave fairly distinctive colorings though not so marked as Uschinsky's medium in starch jelly.

The use of litmus lactose in preliminary isolation work was of some importance in differentiating non acid producing organisms. We have used this medium in deep shake culture with only  $\frac{1}{3}$  the amount of agar viz. 5 to 6 grams, in the ascertaining of the production of acid and gas. We have likewise used in this work the mixture of the following sugars, etc., in testing production of gas and acid in deep shake culture and find it of some importance. viz.

Saccharose	2 grams
Lactose	2 "
Dextrose	2 "
Maltose	2 "
Mannite	2 "
Glycerine	3 cc.
Peptone	10 grams
Extract of beef	3 "
Sodium Chloride	2 "
Agar	5-6 "
Water	1000 cc.
NaOH (and Litmus) to make neutral.	

This medium is not a delicate test for gas production, but it is extremely advantageous. We see no reason why a mixture of the above sugars, etc. should not be used in solution in fermentation tubes for determining in preliminary work the production of gas. It is hardly probable that the presence of a certain sugar to the extent of .2% would inhibit the formation of gas from another sugar. So, for rapidity in determining the biochemical characters of an organism, it may be useful.

### SUMMARY

1. In the foregoing work is shown the relationship existing between the so called "streak" disease of the sweet pea (*Lathyrus odoratus*) and a pathogenic bacterium herein described and named *Bacillus lathyræ* n. sp.

2. The disease is most active during the flowering period of the host, at times becoming so disastrous as to entirely destroy the crop. The season of heavy dew appears to be a time which favors infection.

3. The pathogen produces somewhat similar diseases on the clovers (*Trifolium* spp.) on culinary beans (*Phaseolus* spp.) and on soy beans (*Soja* spp.) altho on the latter only one variety shows much injury among the many grown at the Experiment Station Farm, Newark, De<sup>l</sup>.

4. The disease is widespread in its distribution. In England it has received much comment, being popularly spoken of as the "mysterious" and "dreaded streak" disease. Specimens have been received from England, Massachusetts, Maine, New York, and Delaware. The disease is reported in Ireland.

5. Massee and Chittenden (England) have assigned *Thielavia basicola* as the causal organism. Dyke has referred it to a *Macrosporium*. The foregoing work and work done by my associate J. J. Taubenhau<sup>s</sup> indicate that the two fungi above mentioned are in no way related to "streak" of the sweet pea.

6. The parasitic bacterium is a yellow rodshaped organism having rounded ends, and in size somewhat smaller than the average. It is peritrichiate in the distribution of its flagella; ordinarily it moves rapidly in liquid media.

7. Cross inoculation on the several hosts with different sources of the organism, together with morphological, cultural and biochemical studies on 32 different sources indicate the organism to be a pathogen heretofore not described.

8. Under the numerical system of noting the salient features of an organism *Bacillus lathyri* n. sp. becomes B. 211.2222522.

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## DESCRIPTION OF PLATES

PLATE I. Longitudinal section from stem of red clover through a beginning lesion infected with the "streak" organism (*Bacillus lathyri*, n. sp.) of sweet pea. The organism in the stems of clover penetrate only a few cells in depth, seldom apparently reaching the woody tissue.

Drawn from a photomicrograph printed on glossy Velox, and partly filled in by the aid of the microscope. The background was washed out with a solution of potassium cyanide. X330.

PLATE II. Cross section of the stem of sweet pea through a beginning lesion of the "streak" disease caused by *Bacillus lathyri*. The lesion gradually sinks into the stem until the woody tissue is reached. The organisms do not appear to enter the vessels. The mesophyll is gradually broken down, the lesion extending along the entire stem, producing familiar "streak" effect.

The groups of bacteria show as darkened areas in the mesophyll at points marked B. Photomicrograph X75.

PLATE III. Photograph from a colored plate showing typical symptoms of the "streak" disease in various degrees of progress on sweet pea. The disease is not limited to the stems, but occasionally attacks the foliage, producing water-soaked areas and blight similar to that of the bean disease. The pods are also attacked, causing dark purple lesions which cannot be mistaken for other diseases of the sweet pea. In advance stages of infection the plants succumb to the attack, dying prematurely.

From a drawing by Miss Martha Chamberlin.

PLATE IV. Showing a "streak" disease of Lima bean caused by *B. lathyri*. Extensive cultures were run from lesions on stems of beans with the result that *B. lathyri* was found as the infesting organisms. The organism from this source when inoculated onto red clover produced infections and lesions identical with that of the organism from clovers or sweet pea.

Photographed natural size.

PLATE V. In this plate are shown the typical symptoms of the bean blight disease caused by *Ps. phaseoli* as described by Dr. Erwin F. Smith. On the leaves are formed large water-soaked areas which, upon drying up, produce the characteristic blight. The pods likewise show large water-soaked lesions which sink in and turn brown or even purple when the infection is shallow. Introduced here for comparison with the "streak" disease. Slightly enlarged in photographing.

PLATE VI. Showing the "streak" disease produced by *B. lathyri* on alsike clover. The plant at the left shows dark lesions throughout the stem; the

petioles become so badly diseased as to blight and dry up causing wilting of the leaves. The plant at the right is healthy and used here for comparison.

Reduced to  $4/5$  natural size.

PLATE VII. Comparing "streak" infected stems of red clover (three stems) at the left with healthy stem at the right. The blighted leaf at the left is brought about by the disease attacking the petiole.

Enlarged to 1 1-2 times nat. size.

PLATE VIII. Showing progress of the "streak" disease on red clover. The stem at the left is healthy; the stem next to the one at the left shows beginning lesions; the three stems to the right show further progress of the disease.

Enlarged  $1\frac{1}{3}$  times natural size.

PLATE IX. Showing the type of leaf infection produced by *B. lathyri* on Mammoth clover. The three leaves to the right and below show different stages in the progress of the disease. The healthy leaf at the left upper corner is introduced for comparison.

Two-thirds natural size.

PLATE X. Showing artificial infections produced in laboratory on red clover by use of sources of *B. lathyri* respectively from Red Clover, Alsike clover, Sweet Pea from England and Sweet Pea from Delaware. The types of infection are similar in each case. Previous to infection the plants were thoroly disinfected with potassium permanganate, then after 24 hours carefully washed off with sterile water, following which they were placed under bell jar to note whether any natural infection would arise. The plants were then covered by atomizer spray of 48 hour cultures. The infection followed in 5 to 9 days.

Two-thirds natural size.

PLATE XI. Artificial infections produced in the laboratory by use of pure cultures by *B. lathyri* from alsike, alfalfa and soy bean. The method of inoculation was similar to that described under Plate XI.

Two-thirds natural size.

PLATE XII. Artificial infections produced in the laboratory by use of pure cultures of *B. lathyri* from white pole bean, cowpea and soil suspected of carrying the organism.

Two-thirds natural size.

PLATE XIII. Showing soy beans infected in field with the streak disease caused by *B. lathyri*. Only one variety showed much loss from this disease, in which case 10% of the plants were destroyed. The lesions sink deep into the stem near the ground and the plants blow over similar to that in potato attacked with black leg late in the season.

Photograph taken at the Experiment Station Farm, Newark, Del., Oct. 1912.

PLATE XIV. Showing "streak" lesions on pods of soy bean and also on the lower part of the stem in the plant at the left.

Slightly reduced in photographing.

PLATE XV. Showing lesions of the "streak" disease on the stems of soy beans. When the lesion becomes deep the stems are so weakened that the plant blows over very easily. Enlarged 1 1-2 times in photographing.

PLATE XVI. Showing artificial infection on tomato by a yellow bacterium with morphological, cultural, and biochemical characteristics similar to that of the "streak" disease organism. No cross inoculation work was carried out from sweet pea to tomato and vice versa. In the natural infection in field there were associated quite frequently a *Fusarium* and an *Alternaria*. See Plate XVII. Photograph natural size.

PLATE XVII. Showing appearance of fruit rot or "point rot" of tomato from natural infection in field when the surface has been sterilized and the fruit placed in moist chamber; quite frequently there is associated with the bacterium a *Fusarium* and an *Alternaria*. The former may induce some symptoms of rot; the latter is saprophytic as indicated by a series of inoculation work. Photographed natural size.

PLATE XVIII. Showing appearance of the streak disease organism, *B. lathyri* on nutrient agar and nutrient gelatin from Red Clover, two different sources, viz. No. 15 and No. 13a. The colonies on nutrient agar have an early tendency to become stellate.

Reduced to  $\frac{1}{2}$  natural size in photographing.

PLATE XIX. Showing appearance of the "streak" disease organism *B. lathyri* on nutrient agar and nutrient gelatin from sweet pea, bean, and a forage species of *Lathyrus*, sources No. 5, 40 and 26f.

The colonies on nutrient agar have an early tendency to become stellate.

Reduced to  $\frac{1}{2}$  natural size in photographing.

PLATE XX. Showing appearance of the "streak" disease organism *B. lathyri* on nutrient gelatin from sweet pea; sources 7A', 4', and 8'. The colonies on nutrient agar have an early tendency to become stellate.

Reduced to  $\frac{1}{2}$  natural size in photographing.

PLATE XXI. Showing appearance of the "streak" disease organism *B. lathyri* on nutrient agar from Red Clover, Soy bean and an organism with similar morphological, cultural and biochemical characteristics from the point rot of tomato.

The surface colonies on nutrient agar all have an early tendency to become stellate.

Reduced to  $\frac{1}{2}$  natural size in photographing.



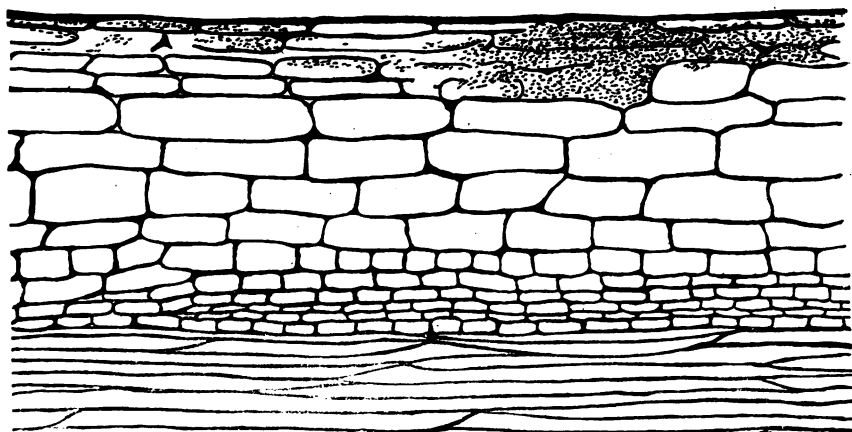


Plate I

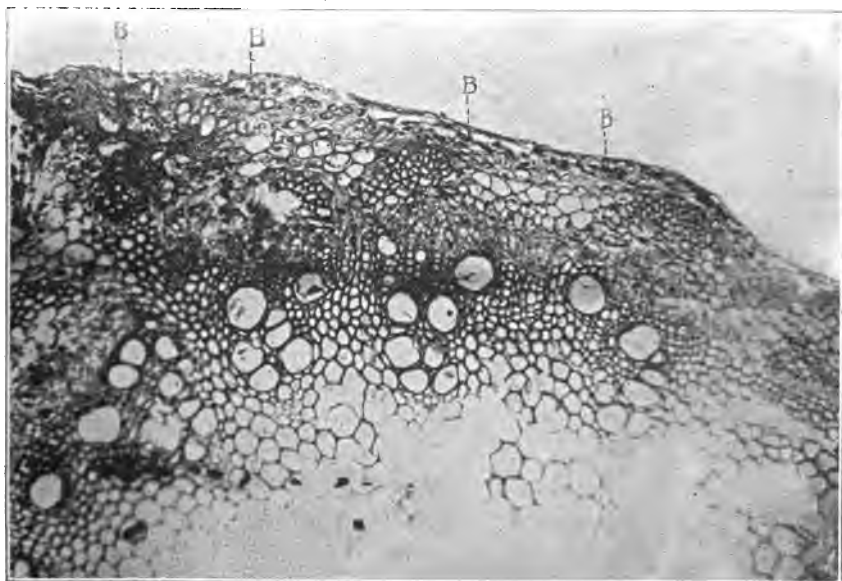


Plate II



Plate III

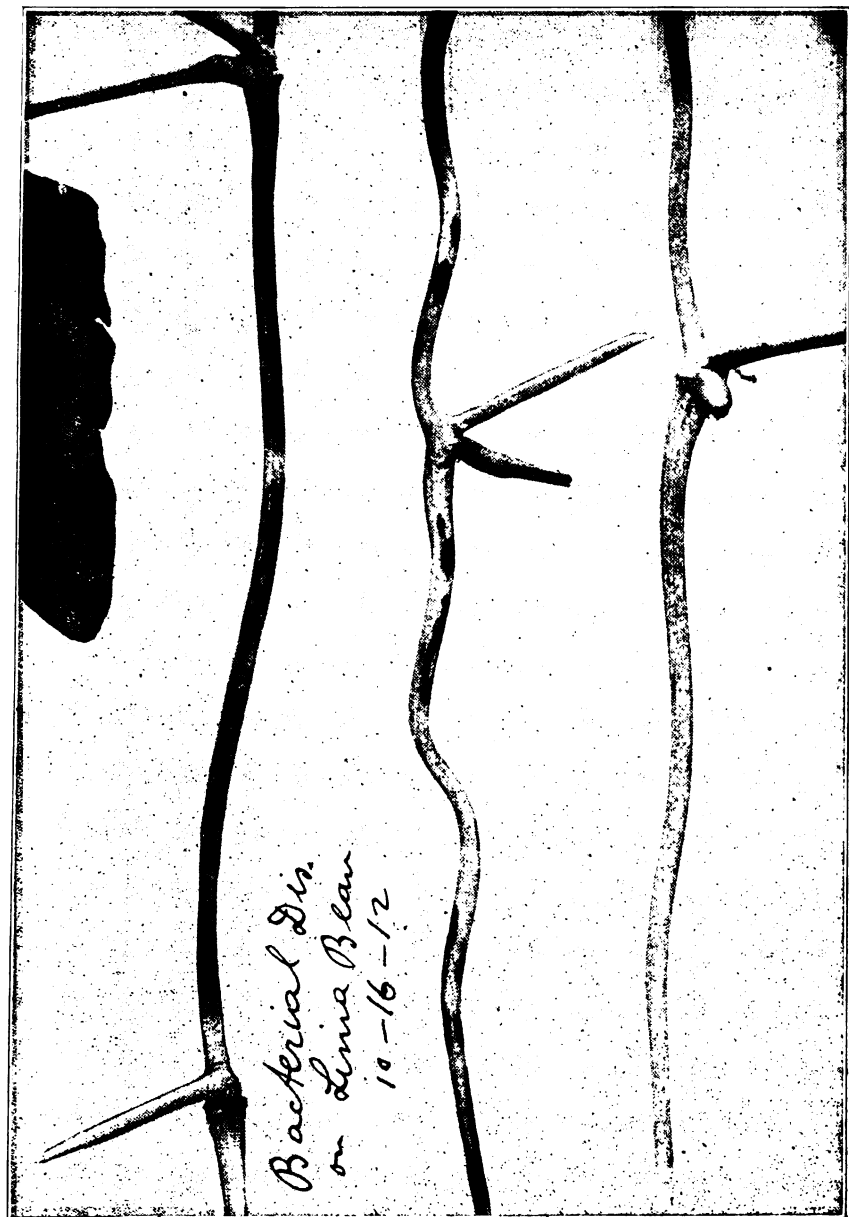


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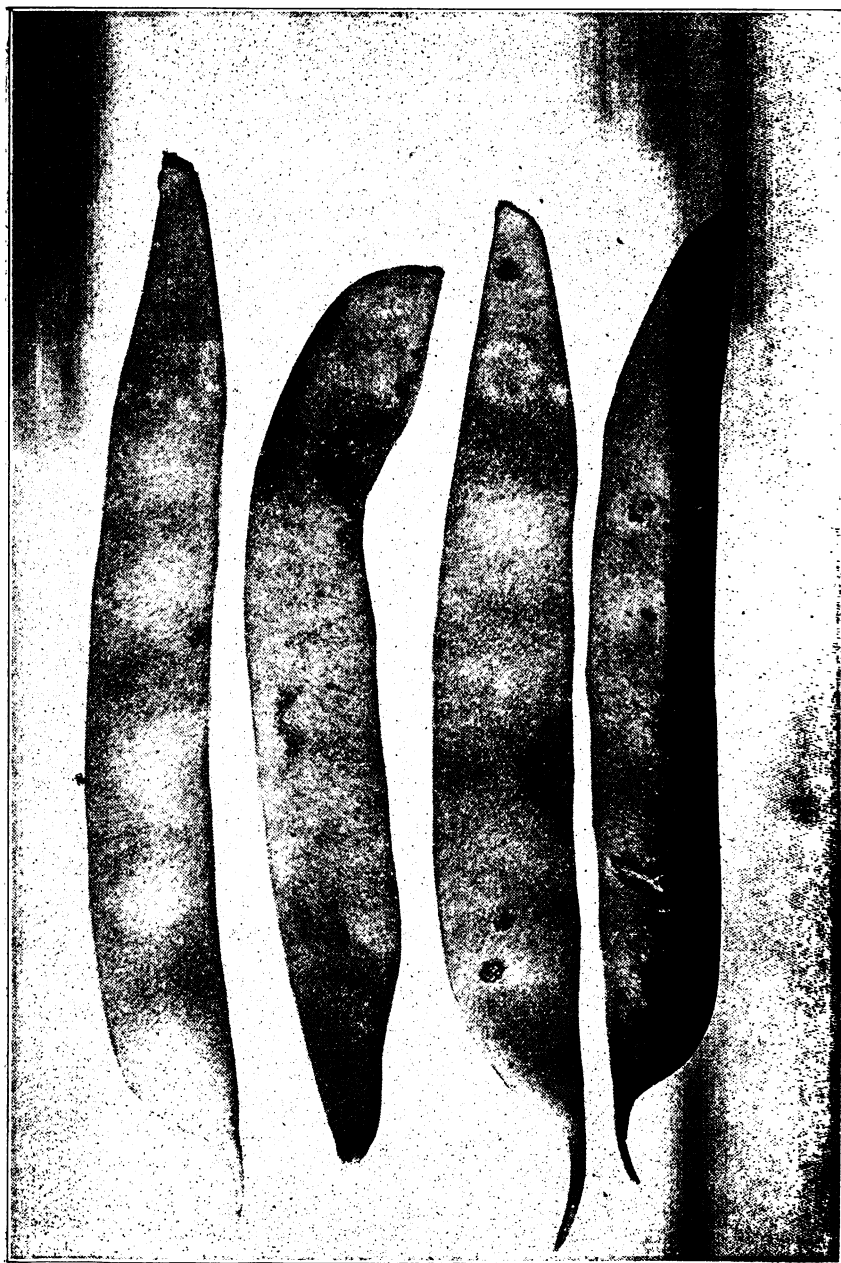


Plate V



**Plate VI**



**Plate VII**

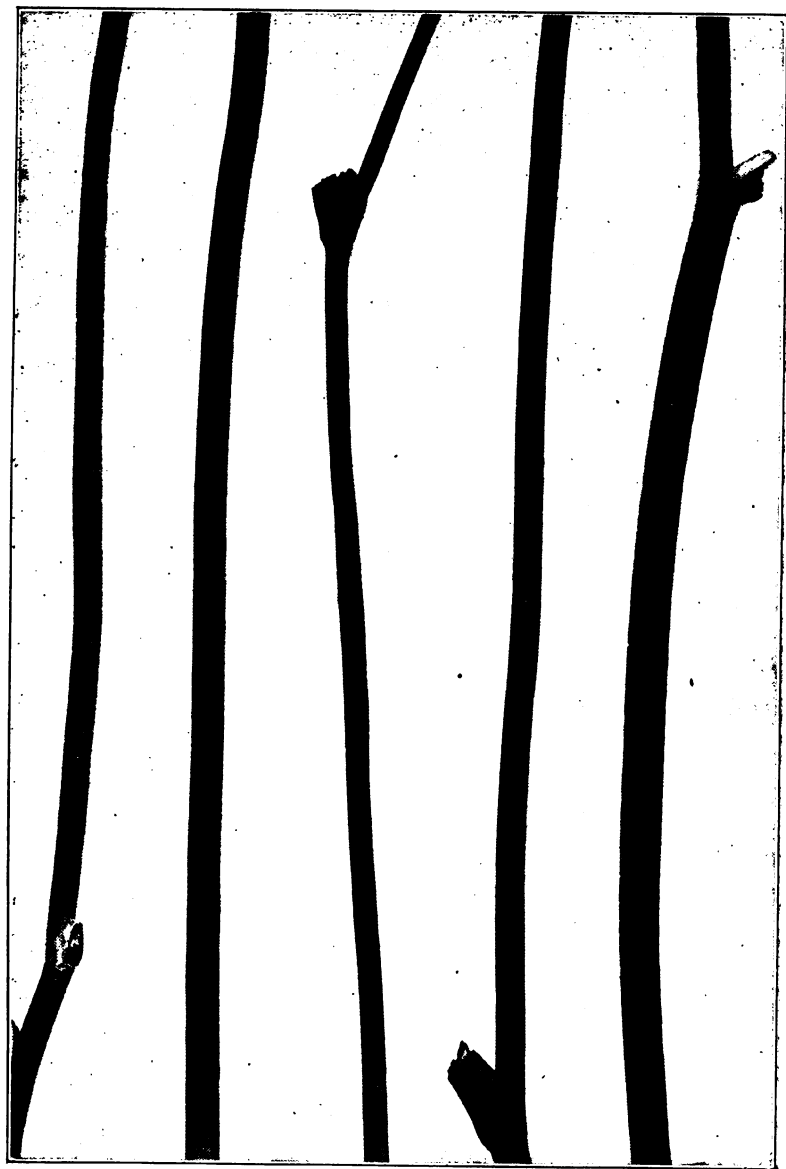


Plate VIII

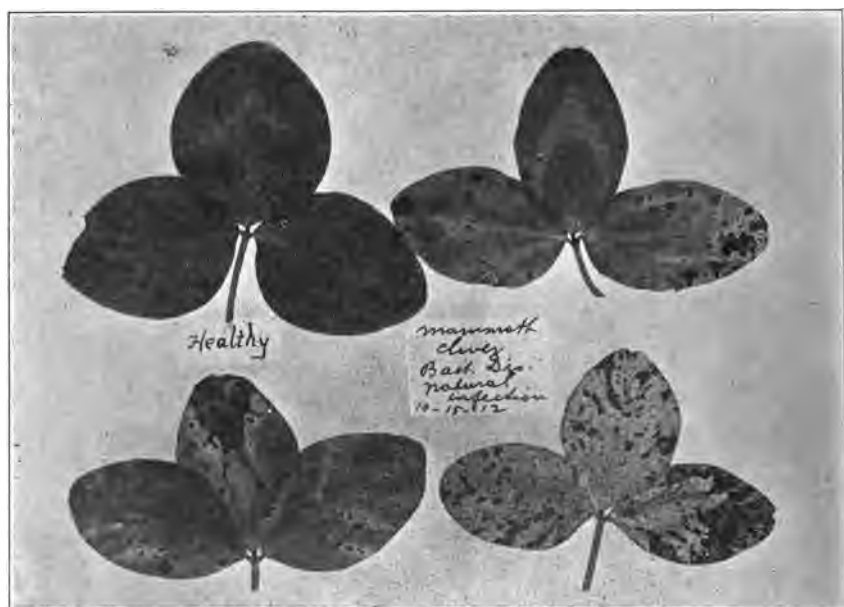


Plate IX

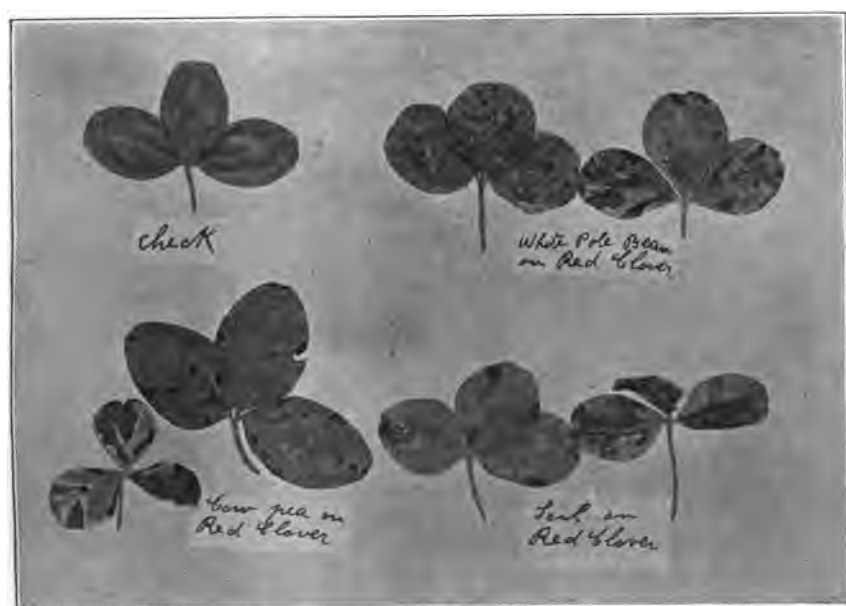




**Plate X**



Plate XI



**Plate XII**



**Plate XIII**



Plate XIV



**Plate XV**



**Plate XVI**

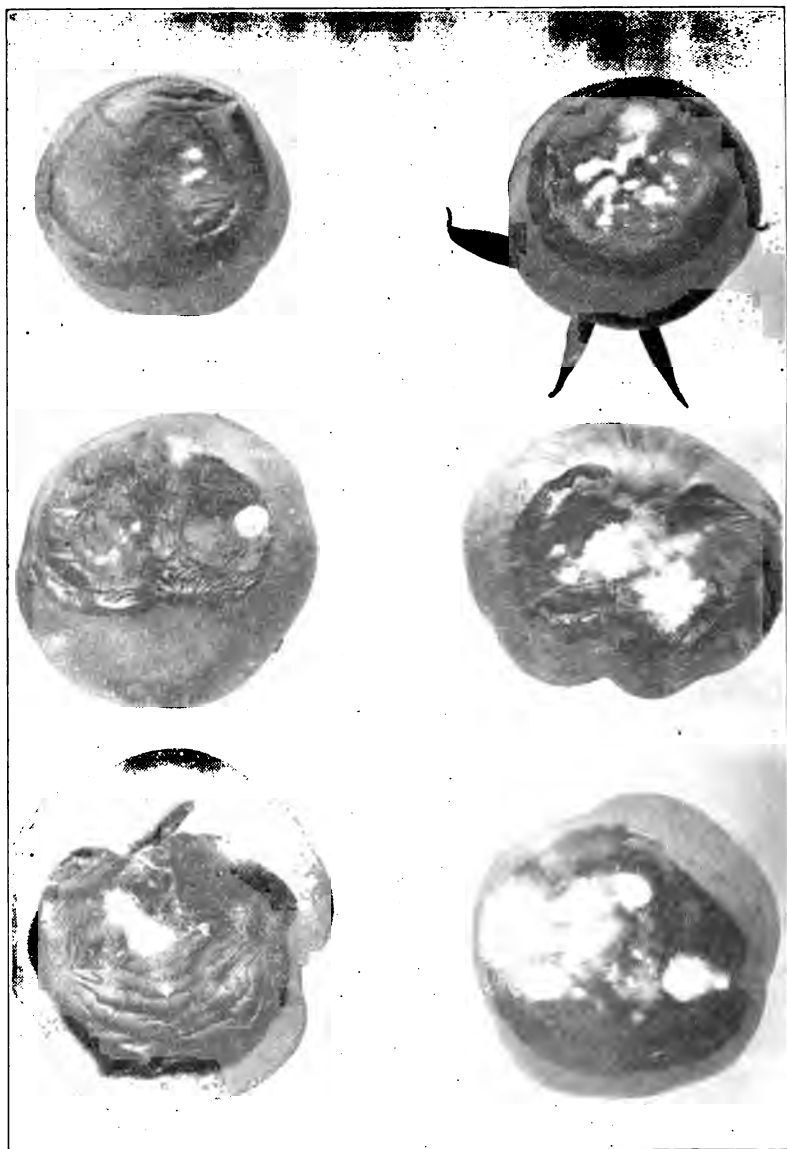


Plate XVII